

APPENDIX B

CLAIMS PENDING IN USSN 09/249,529 WITH ENTRY OF THIS AMENDMENT

1. A method of selecting a polypeptide that is internalized into a target cell, said method comprising:

i) contacting one or more target cells with one or more members of a phage display library displaying one or more polypeptides;

ii) removing and eliminating members of said library that are bound to the exterior surface of said target cells with a strong wash; and

iii) identifying internalized members of said phage display library that are internalized into one or more of said target cells, where said internalized members of said phage display library each display a polypeptide that is internalized into a target cell.

51. The method of claim 1, wherein said method further comprises contacting the target cells with cells of a subtractive cell line.

52. The method of claim 51, wherein said method further comprises contacting the target cells with live cells of a subtractive cell line.

53. The method of claim 1, wherein said removing comprises contacting the target cells with a low pH wash.

54. The method of claim 51, wherein said removing comprises contacting the target cells with a low pH wash.

55. The method of claim 1, wherein said removing comprises contacting the target cells with a trypsin.

56. The method of claim 51, wherein said removing comprises contacting the target cells with a trypsin.

57. The method of claim 51, wherein the target cells are cells that are transformed a nucleic acid that encodes and expresses a target receptor and the subtractive cell line is the non-transformed cell line.

2. The method of claim 1, wherein said phage display library is an antibody phage display library.
3. The method of claim 2, wherein said antibody phage display library displays single chain antibody Fv regions.
4. The method of claim 1, wherein said identifying comprises recovering internalized phage and repeating steps (i) through (iii) to further select for internalizing binding moieties.
5. The method of claim 4, wherein said recovering comprises:
 - (a) lysing said target cells to release internalized phage; and
 - (b) infecting a bacterial host with said internalized phage to produce phage for a subsequent round of selection.
6. The method of claim 4, wherein said recovering comprises recovering nucleic acids encoding the phage-displayed antibody.
7. The method of claim 1, wherein said identifying comprises detecting expression of a reporter gene or a selectable marker.
8. The method of claim 1, wherein said cells of a subtractive cell line are present in at least 2-fold excess over said target cells.
9. The method of claim 1, wherein said target cells form an adherent layer in said method.
10. The method of claim 1, wherein step (ii) is performed at a temperature lower than step (iv).

11. The method of claim 1, wherein step (ii) is performed at about 4°C.
12. The method of claim 1, wherein said phage express a selectable marker.
13. The method of claim 12, wherein said selectable marker is selected from the group consisting of a fluorescent protein, an antibiotic resistance gene, and a chromagenic gene.
14. The library of claim 13, wherein said chromagenic gene is selected from the group consisting of horse radish peroxidase, β -lactamase, luciferase, and β -galactosidase.
15. The method of claim 1, wherein said target cells are selected from the group consisting of solid tumor cells, members of a cDNA expression library, cells that overexpress a cytokine receptor, cells that overexpress a growth factor receptor, metastatic cells, cells of a transformed cell line, cells transformed with a gene or cDNA encoding a specific surface target receptor, and neoplastic cells derived from outside a solid tumor.
16. The method of claim 1, wherein said cells of a subtractive cell line are selected from the same tissue type as the target cells.
17. The method of claim 1, wherein said cells of a subtractive cell line are selected from the group consisting of fibroblasts, monocytes, stem cells, and lymphocytes.